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(54) Title: COMPOSITION AND METHOD FOR ADMINISTRATION OF BIO-AFFECTING CATALYSTS

(57) Abstract

Compositions and methods for administration of a bio-affecting catalyst, such as an enzyme, are provided. The compositions comprise at least one enzyme or other bio-affecting catalyst, retained within a matrix. The matrix provides a barrier between the entrapped enzyme and external degradative enzymes or cellular defense systems of a patient's body, and also prevents release of the enzyme. The matrix comprises pores or channels to enable entry and exit of substrates and products. Preferably, the matrix is a high viscosity cubic phase of one or more suitable amphiphilic compounds, such as glyceryl monooleate. Preferred methods of the invention comprise formulating the composition as an injectable low viscosity phase, which undergoes phase transition upon exposure to physiological conditions.

COMPOSITION AND METHOD FOR ADMINISTRATION OF BIO-AFFECTING CATALYSTS

FIELD OF THE INVENTION

The present invention relates to treatment of diseases and other pathological conditions associated with enzyme defects or imbalances. In particular, the invention provides compositions and methods for supplying enzymes or other bio-affecting catalysts, for long-term enzyme replacement/augmentation therapy.

BACKGROUND OF THE INVENTION

To date, more than one hundred and fifty metabolic diseases have been ascribed to specific enzyme defects. These defects often result in physically and mentally crippling diseases. In many of the defects a specific enzyme is completely missing, while in others the enzyme is replaced with a relatively inactive isoenzyme. A small portion of the enzyme deficiency diseases can be treated by controlled diets (e.g., phenylketonuria, a defect in aromatic amino acid metabolism, can be treated by abstaining from foods containing phenylalanine). A potentially more generally applicable treatment strategy, however, is enzyme replacement/augmentation therapy wherein the missing enzyme is administered to a patient in order to alleviate or remove the symptoms of the disease.

In conventional enzyme replacement therapy, an exogenous enzyme is administered to the patient either intravenously or intramuscularly. One problem with the conventional therapy arises because enzymes are proteins and are therefore susceptible to rapid proteolytic degradation as well as elimination from the patient's body. Additionally, the exogenous enzyme may elicit undesirable immunological responses upon being introduced into the body of the patient. As a result, the patient's own defenses may actually attack and destroy the enzyme,

the periodontal pocket. U.S. Patent No. 5,151,272 to Engstrom et al. discloses a controlled-released composition for biologically active materials which comprises a gel-like liquid crystalline phase (e.g., lamellar, hexagonal, cubic and micellar). The biologically active substance is released into the body as a result of erosion of the liquid crystal phase or diffusion of the substance into the surrounding medium.

The above-described gels and liquid crystal matrices are all designed to deliver biologically active agents by releasing the agent into the body fluid or tissue into which the composition has been injected, implanted or otherwise administered. However, for enzyme replacement therapy, as discussed above, release into the body is detrimental for two reasons: first, it exposes the enzyme to proteolytic and immunological attack; second, it allows the enzyme to diffuse from the precise location in the body where it may be required. What is more, because enzymes are catalysts, their release into the body from a gel or liquid crystal matrix is unnecessary. It is necessary only that the enzyme be accessible to its appropriate substrates in the body, and that it be capable of exerting its required enzymatic function and releasing the enzymatic products back to the body where they are required.

Thus, it would be a significant advance in the art of enzyme replacement therapy to provide compositions and methods for supplying an enzyme (or other biologically relevant catalyst) to a patient in a form in which the enzyme is protected from proteolytic degradation and immunological attack, yet is accessible to entry and exit of substrates and products and is capable of performing the requisite enzymatic activity required by the patient. It is also desirable for such compositions and methods to provide therapeutic concentrations of a required enzyme in a localized region of the body. Preferably, the compositions should be

the bio-affecting catalyst from external proteolytic or other degradative enzymes and/or cellular defense mechanisms present in the physiological environment. Fluid communication through the matrix occurs as a result of the composition and structure of the matrix. High viscosity gel matrices comprise amorphous networks of polymeric molecules, the spaces therebetween providing channels for the fluid communication. High viscosity liquid crystalline matrices comprise semi-regular or regular arrays of amphiphilic molecules, separated by pores or channels for the fluid communication. Liquid crystalline phase high viscosity matrices are preferred in the present invention, with cubic phases being particularly preferred.

The above-described composition preferably is prepared as a low viscosity phase mixture comprising the bio-affecting catalyst and at least one matrix-forming compound, and can be induced to undergo a phase transition to a high viscosity phase to produce the matrix with the catalyst retained therein. The phase transition is inducible by exposing the low viscosity phase to changes in one or more conditions, including temperature, pH or solvent composition. In a preferred embodiment, the phase transition is inducible by introduction into the body of a patient.

According to another aspect of the present invention, a composition is provided that comprises at least one bio-affecting catalyst retained within a high viscosity cubic phase matrix. The composition is prepared as a low viscosity phase, comprising by weight between about 60% and about 99.99% of glyceryl monooelate, up to about 40% (more precisely, 39.99%) of an aqueous phase, and between about 0.01% and about 40% of one or more bio-affecting substances.

According to another aspect of the present invention, a method is provided for supplying a bio-affecting catalyst to a patient. The method comprises

Fig. 2 is a plot of the percent of horseradish peroxidase (HRP) activity as a function of time in the presence of lipase and/or trypsin; and

Fig. 3 is a plot of the percent of catalase activity as a function of time.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

In its most basic form, the composition of the present invention comprises a matrix in which is disposed a bio-affecting catalyst, such as an enzyme. The composition is initially formed as a mixture of the matrix-forming compound(s) and bio-affecting catalyst(s) wherein the mixture exists as a low viscosity phase. However, the mixture is capable of undergoing a phase transition to a high viscosity phase, such as a liquid crystal or gel, with the bio-affecting catalyst entrapped within. The high viscosity phase matrix comprises pores or channels that allow for the ingress and egress of substrates and products to and from the catalyst disposed therein. However, the size of the pores or channels is sufficiently small to impede or prevent diffusion of the catalyst from the matrix, and to protect the catalyst from proteolytic enzymes and cells of the immunological response system.

The present invention can be practiced with a variety of bio-affecting catalysts. These catalysts may be naturally-occurring enzymes or enzyme derivatives, or they may be any other catalyst (e.g., catalytic peptides or peptide derivatives) useful for producing biological products normally supplied by a naturally-occurring enzyme in a patient's body. Enzyme classes that may be utilized in the present invention include, but are not limited to, oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases (or synthetases). Examples of enzymes suitable for use in the present invention, include but are not limited to, the enzymes set forth in Table "A" below.

As described above, the matrix possesses pores or channels that allow for the movement of substrates and products to and from the enzyme and the external environment. The pores or channels are sufficiently narrow to prevent or impede the bio-affecting catalyst from diffusing out of the matrix. Retention may also be facilitated by partitioning of the catalyst with the lipid phase of the matrix (preferred matrices of the invention comprise a lipid phase). Under certain circumstances (e.g., for catalysts with low molecular weights or for small co-factors), it may be desirable to further restrict the rate at which the bio-affecting catalyst diffuses from the matrix. This may be accomplished by attaching the catalyst to (or combining it with) an anchor molecule that increases the molecular weight of the catalyst without materially affecting its function, or that increases the affinity of the catalyst for the matrix (e.g., by facilitating partitioning with a lipid phase of the matrix). Examples of anchor molecules include, but are not limited to: l-palmitic acid and other fatty acids, peptides, proteins, polyethylene glycol and other polymers, or a combination of such molecules.

The bio-affecting catalyst is disposed within a matrix comprising pores or channels of a size that enable entry/exit of substrates/products, while concomitantly preventing exit of the bio-affecting catalyst during its functional lifetime and substantially preventing entry of proteolytic or other enzymes or immunological defense cells of the body. The pore or channel size may vary, but is preferably within the range of 0.1 to 100 nm, more preferably between 1 and 20 nm, and most preferably on the average of 5 nm. As discussed in greater detail below, the matrix preferably comprises a relatively uniform channel structure, that may be adjusted by varying the composition and relative concentrations of components used to prepare the matrix. Optimum size

and references cited therein (e.g., U.S. Patent Nos. 4,188,373, 4,474,751, 4,474,752, 5,292,517 and 5,252,318). The high viscosity phase formed by these reversibly-gelling compositions are gels comprising amorphorous polymer networks in a substantially aqueous environment. Preferred gels comprise low molecular weight polymers (e.g., mw < 50,000), which will slowly dissolve in the body, or polymers that are otherwise degradable in the body, to eliminate the need for surgical removal.

Although gels can be used, it has been discovered in accordance with the present invention that matrices having well structured pores or channels provide better accessibility of the bio-affecting catalysts to substrates (and better release of products) than do gel matrices with amorphous polymer networks, or other matrices having poorly formed channel structures. Accordingly, preferred for use in the present invention are compounds capable of undergoing a phase transition to a liquid crystal structure, such as a cubic, hexagonal or reverse hexagonal phase. Of these, cubic phases are most preferred because of their uniform pore/channel structure.

Compounds that may be induced to undergo phase transitions to liquid crystal phases are known in the art (see, e.g., Fontell, Colloid and Polymer Science 266: 264-285, 1990). Liquid crystalline phases are common in amphiphilic lipid systems, including compositions comprising simple surfactants, straight- or branched-chain lipids and/or complex biological lipid mixtures. Some amphiphilic lipid systems are capable of undergoing a phase transition, e.g., upon temperature increase from a low viscosity lamellar phase (i.e., a phase formed by lipid-water systems in which lipid bilayers alternate with water layers) to a high viscosity liquid crystalline phase. The liquid crystalline phase may be a cubic phase (i.e., an isotropic phase with a three-dimensional

3. bio-affecting catalysts, co-catalysts and/or co-factors: in appropriate effective concentrations, preferably about 0.01% - 50% (but could be less or greater depending on the specific catalysts and other substances used);

4. other ingredients: approx. 0.01% - 25%.

Amphiphilic molecules that may be utilized in preparing compositions of the invention include, but are not limited to: lipids, surfactants and soaps capable of forming high viscosity liquid crystalline phases. These components may be used alone or in combination.

Nonlimiting examples of lipids that may be utilized in the compositions of the invention include glycerides, phospholipids, fatty acids and sphingolipids. Glycerides include, but are not limited to, monoglycerides, diglycerides and triglycerides. In a preferred embodiment the monoglyceride, glyceryl mono(cis-9)oleate (also referred to as glyceryl monooleate), is used, as described in greater detail below.

Nonlimiting examples of soaps that may be incorporated into compositions of the invention include anionic and cationic soaps. Examples of surfactants include, but are not limited to, nonionic and zwitterionic surfactants.

Nonlimiting examples of suitable aqueous media to be used in formulating the composition of the invention include water, various biologically acceptable buffers (e.g., phosphate buffers, Tris buffers and other similar physiological buffers) and isotonic solutions containing salts. In some embodiments, the aqueous medium may contain a biologically acceptable alcohol, such as ethanol, for purposes of improving the solubility of polymers in the low viscosity phase (see, e.g., U.S. Patent Application No. 08/261,731, the disclosure of which is incorporated herein by reference).

dissolves, or (3) the matrix erodes by a combination of dissolution, degradation and/or other means. An erodible matrix eliminates the need for removal of the composition upon completion of the treatment. If an erodible matrix is utilized, the rate of erosion should be adjusted so that the matrix erodes substantially after the effective life span of the bio-affecting catalyst is completed. In a preferred embodiment, the rate of lipid degradation can be delayed by adding lipase inhibitors to the matrix-forming mixture in its low viscosity phase. The inclusion of lipase inhibitors functions to reduce to activity of lipases that may attack the lipid-containing matrix of the composition. As a result, the useful lifetime of the compositions are increased.

An exemplary composition of the invention comprises, as a matrix-forming compound, the polar lipid, glyceryl monooleate (hereinafter GMO), also known as monoolein. At 0-40% (preferably 10-20%) (by weight) water in GMO, the mixture forms a low viscosity, opaque lamellar phase in which bio-affecting catalysts and other ingredients may be solubilized. The viscosity of the lamellar phase is low enough that the GMO-catalyst mixture can be passed through a syringe. Upon injection into a patient's body, the mixture undergoes a phase transition to a high viscosity, clear cubic phase (approximately 10^5 centipoise, or greater). The phase transition is induced by the absorption of water and increase in temperature (from room temperature to body temperature). The cubic phase gradually erodes in the body, eliminating the need for surgical removal.

The GMO cubic phase comprises aqueous channels with average diameters of about 5 nm, which allow substrates and product to migrate to and from the bio-affecting catalyst (which, in this preferred embodiment, is a naturally-occurring enzyme or combination of enzymes). In addition, the cubic phase stabilizes the enzyme and protects the enzyme from proteases,

ordered liquid crystalline phase. In one embodiment, the phase transition is induced *ex vivo*, and the resultant matrix-enzyme composition is administered to the patient by implantation into the desired location of the patient's body.

In a preferred embodiment, the mixture is administered to the patient by placing the mixture in a syringe and injecting it into the patient intramuscularly, subcutaneously or into a selected tissue. The phase transition is induced by the physiological conditions within the patient's body, including increased temperature and exposure to water. In this embodiment, the mixture also may be injected into a predetermined region of the body, so as to provide increased concentrations of the enzyme or other bio-affecting catalyst in that area.

The general method described above is applicable not only to enzyme replacement/augmentation therapy, but may also be used for treatment of substrate-dependent tumors. One example of a substrate-dependent tumor is human acute lymphoblastic leukemia. Lymphoblastic leukemia cells are deficient in L-asparagine synthetase and depend on exogenous supplies of L-asparagine formed in surrounding normal cells. Current therapy for lymphoblastic leukemia involves intravenous administration of the enzyme, L-asparaginase. L-asparaginase degrades L-asparagine, thereby depleting the body of L-asparagine. This causes the malignant cells to die of starvation due to lack of adequate supply of L-asparagine, which is an essential amino acid. Intravenous administration of L-asparaginase is not particularly effective because the enzyme is rapidly cleared from the bloodstream and also elicits undesirable immunological responses in the patient's body.

The compositions of the invention may provide a more effective treatment for substrate-dependent tumors. For example, injection of a composition comprising L-

EXAMPLES

In order to demonstrate the efficacy of the compositions and methods of the present invention, mixtures of a matrix and various enzymes or complex enzyme systems were prepared. Myverol™ 18-99 glyceryl monooleate (GMO) (obtained from Eastman Chemical Company), a high quality distilled monoglyceride containing a minimum of 90 % assay as a monoester, was used as the matrix. The enzyme systems tested include horseradish peroxidase, catalase, and a combination of human cytochrome P450-2A6, cytochrome P450 reductase, and glucose-6-phosphate dehydrogenase.

Examples 1-7
Horseradish Peroxidase

Example 1: Phase Change

The activity of horseradish peroxidase (hereinafter, HRP) was tested using 2,2'-azino-bis-[3-ethylbenzthiazoline-6-sulfonic acid] (hereinafter, ABTS) as the substrate. In the presence of H_2O_2, HRP oxidizes ABTS to a radical cation which is deep bluish-green in color and has an absorption maximum at 410 nm. The concentration of product in solution is measured using spectrophotometric analysis. In addition, HRP concentrations in solution can be determined using fluorescein isothiocyanate-labelled HRP (hereinafter, HRP-FITC), which can be detected fluorimetrically at an excitation wavelength of 490 nm and an emission wavelength of 515 nm.

The samples of lamellar and cubic phase GMO containing HRP (or HRP-FITC) used in the examples were prepared as follows. A 10 mM solution of phosphate buffer (pH 7.4) containing HRP was mixed with GMO such that the final composition was, in weight percent, 85.00% GMO, 14.87% buffer, and 0.13% HRP. The mixture was stored at room temperature for 24 hours to form the

that the extent and rate of escape of entrapped molecules appears to be molecular weight dependent (HRP (44,000 Da) < Insulin (6,000 Da) < Methylene blue (374 Da)). Thus, high molecular weight enzymes will tend to be better entrapped in the cubic phase without the use of anchor molecules.

Table 1

Time (days)	Fraction Escaped		
	HRP-FITC	Insulin-FITC	Methylene Blue
0	0.000	0.000	0.000
0.08	0.030	0.060	0.120
0.17	0.050	0.083	0.150
0.50	0.076	0.130	0.260
1	0.100	0.200	0.392
1.5	---	---	0.460
2	0.149	0.280	0.530
3	---	---	0.650
4	0.200	0.389	0.740
5	---	---	0.820
6	0.245	0.483	0.920
8	0.279	0.563	0.960
10	0.310	0.630	0.973
14	0.370	0.750	0.980
18	0.419	0.850	---
22	0.460	0.930	---

GMO layer absorbed some of the buffer solution and transformed into the clear cubic phase on top of the cubic layer containing HRP. The remaining buffer solution was removed and 4 mL of a substrate solution containing 0.25 mM ABTS and 0.05 mM H_2O_2 was added to the vial. After some time, the HRP containing layer turned deep bluish-green while the upper cubic phase layer remained colorless. As more time elapsed, the substrate solution on top of the upper cubic phase layer also acquired a bluish-green color. Those results clearly show that the ABTS from the substrate solution diffused through the upper cubic phase layer to the cubic phase layer containing HRP and reacted with the entrapped HRP. The bluish-green colored product then diffused through both of the cubic phase layers into the substrate solution.

Example 5: Enzyme Assays

An assay procedure was developed to test the activity of HRP immobilized in the cubic phase. The immobilized HRP assay involved placing 1 gram of a cubic phase containing HRP at the bottom of a glass vial (12 mm inner diameter). The cubic phase was thoroughly rinsed with buffer solution to remove any surface associated HRP. A 2 mL aliquot of a substrate solution containing 0.25 mM ABTS and 0.05 mM H_2O_2 was poured on top of the cubic phase in the vial and allowed to react for 2 minutes with continuous shaking. At the end of the 2 minutes, the reaction was stopped by decanting the substrate solution into another vial containing 2 mL of a solution containing 2% dodecyl sulfate and 0.1% sodium azide. The concentration of the products was measured spectrophotometrically by determining the absorbance of the solution at 410 nm following appropriate dilution.

A similar assay procedure was used to test the activity of HRP in free solution. The free HRP assay involved preparing a solution of HRP in a 10 mM phosphate

Table 2

Time (days)	Activity of Free HRP	Activity of Immobilized HRP
0	100.0%	100.0%
7	33.0%	51.8%
15	1.7%	23.1%
30	0.0%	4.7%
60	0.0%	1.7%
90	0.0%	0.6%

The results in Table 2 clearly show that the activity of HRP in free solution decreases rapidly with time and is completely lost within 30 days. However, the HRP immobilized in the cubic phase continues to exhibit activity even beyond 90 days. The activity measurements for HRP immobilized in the cubic phase may underestimate the true activity of HRP in the cubic phase since the buffer solution in which the cubic phase was stored was not tested for HRP activity and may contain some HRP which slowly diffused from the cubic phase.

Example 7: Effect of Lipase and Protease Enzymes on Immobilized Enzyme Activity and Matrix Structure

When a GMO-based composition of the invention is injected into a patient's body, the cubic phase formed will be exposed to protease and esterase (lipolytic) enzymes circulating in the body. These enzymes are capable of degrading the entrapped enzyme and the cubic phase, respectively. The effects of trypsin (a protease enzyme) and lipase (an esterase enzyme) on the activity of HRP immobilized in the cubic phase and the cubic phase structure itself were therefore investigated. Twelve aliquots, each containing 1 gram of the cubic phase

Table 4

Time (days)	Group 1 (buffer)	Group 2 (trypsin)	Group 3 (lipase)	Group 4 (t + l)
	Weight (%)	Weight (%)	Weight (%)	Weight (%)
0	100.00	100.00	100.00	100.00
1	103.27	103.40	110.43	111.63
2	103.67	103.87	108.20	106.57
3	103.57	103.47	100.60	101.43
5	103.30	103.10	94.40	96.70
7	102.80	102.60	88.57	91.60

The activity measurements in Table 3 are plotted in Fig. 2. The data clearly show that only about 33% of the initial HRP activity remains when HRP in free solution is exposed to trypsin for 1 day. In contrast, HRP immobilized in the cubic phase exhibits about 85% activity after 1 day and about 50% activity after 7 days. Further, the observed activity for HRP immobilized in the cubic phase is virtually unaffected by exposure to trypsin, indicating that the cubic phase protects the entrapped enzyme from degradation by protease enzymes in the buffer solution.

The data in Table 4 show that aliquots exposed to the lipase enzyme showed a gradual loss in weight over time, with approximately an 8-12% loss at the end of 7 days. This suggests that the lipase enzymes in the body will slowly biodegrade the cubic phase.

Example 8

Catalase

Catalase catalyzes the breakdown of hydrogen peroxide (H_2O_2) to water and oxygen. H_2O_2 absorbs light at a wavelength of 240 nm and, therefore, catalase activity

Table 5

Time (days)	Activity of Free Catalase (%)	Activity of Immobilized Catalase (%)
0	100.00	100.00
1	90.07	92.08
3	62.48	78.83
7	33.06	73.02
10	8.23	57.34
14	1.32	49.15

The results in Table 5 and Fig. 3 clearly show that catalase is successfully immobilized in the cubic phase with retention of activity. A 50% decrease in the activity of the immobilized catalase is observed over a 14 day period. In comparison, the activity of the free catalase in solution decreased by about 99% in 14 days. In addition, the absence of catalase in the buffer solution suggests that catalase (250,000 Da) does not diffuse through the aqueous channels in the cubic phase.

Example 9

Human Cytochrome P450

The cytochrome P450 family consists of membrane bound enzymes that metabolize many chemicals in the body. CYP-2A6 is a coumarin 7-hydroxylase enzyme: it converts coumarin (substrate) to 7-hydroxycoumarin (product). The rate of formation of the product can be measured fluorometrically with excitation at 390 nm and emission at 440 nm. In this example, it was determined whether a complex enzyme system comprising CYP-2A6, CYP-r and glucose-6-phosphate dehydrogenase (G6PDase) could be immobilized in a GMO cubic phase with retention of enzymatic activity.

Table 6
Cytochrome P-450 2A6 Activity (n = 2)

Time	Activity of free CYP-2A6 in solution at 37°C			
(hours)	Average Activity	Std. dev.	% Activity	Std. dev.
	(pmole/min x mg protein)			
0	259.98	8.28	100.00	3.18
3	182.47	16.88	70.19	6.49
6	95.33	3.39	36.67	1.31
18	57.76	3.87	22.22	1.49
48	14.96	1.16	5.75	0.45
84	1.64	0.36	0.63	0.14

Time	Activity of immobilized CYP-2A6 at 37°C			
(hours)	Average Activity	Std. dev.	% Activity	Std. dev.
	(pmole / min x g gel)			
0	1.43	0.36	100.00	25.11
3	2.02	0.54	141.79	37.84
6	1.40	0.05	97.76	3.72
18	1.18	0.02	82.80	1.09
48	1.43	0.24	100.42	16.99
84	1.13	0.35	79.05	24.32

WHAT IS CLAIMED IS:

1. A composition for administration of a bio-affecting catalyst, said composition comprising at least one said bio-affecting catalyst retained within a matrix, said matrix substantially preventing release of said catalyst for a pre-determined time period, said matrix enabling fluid communication between said catalyst and an external medium in which said composition is disposed, said fluid communication resulting in accessibility of said catalyst to substrates disposed within said external medium, and release into said external medium of products formed by said catalyst.

2. The composition of claim 1, wherein said bio-affecting catalyst is selected from the group consisting of enzymes, enzyme derivatives, peptides and peptide derivatives.

3. The composition of claim 1, wherein said bio-affecting catalyst is combined with an anchor molecule.

4. The composition of claim 1, which erodes substantially after expiration of said pre-determined time period.

5. The composition of claim 1, which is formable as a low viscosity phase mixture comprising said bio-affecting catalyst and at least one matrix-forming compound, and is inducible to undergo a phase transition to a high viscosity phase to produce said matrix with said bio-affecting catalyst retained therein.

6. The composition of claim 5, wherein said phase transition is inducible by exposing said low

amphiphilic matrix-forming compound and said at least one bio-affecting catalyst.

15. The composition of claim 14, wherein said amphiphilic matrix-forming compound is selected from the group consisting of lipids, surfactants and soaps.

16. The composition of claim 15, wherein said lipid is a glyceride.

17. The composition of claim 16, wherein said glyceride is glyceryl monooleate.

18. The composition of claim 17, wherein said low viscosity phase mixture comprises by weight between about 60% and about 99.99% glyceryl monooleate, up to about 40% of an aqueous phase, and between about 0.01% and 40% of said bio-affecting catalyst.

19. The composition of claim 18, wherein said low viscosity phase mixture comprises by weight about 85% of glyceryl monooleate, about 14.87% of said aqueous phase, and about 0.13% of said bio-affecting catalyst.

20. A composition for administration of a bio-affecting catalyst, said composition comprising at least one said bio-affecting catalyst retained within a high viscosity cubic phase matrix that substantially prevents release of said catalyst for a pre-determined time period, said cubic phase having fluid communication channels enabling accessibility of said catalyst to substrates disposed within an external fluid medium in which said composition is disposed, and release into said external medium of products formed by said catalyst, said composition being formable as a low viscosity phase mixture comprising said bio-affecting catalyst, and at least one cubic phase-forming compound, said mixture

27. The composition of claim 26, wherein said phase transition is inducible in response to increasing temperature from between about 18-25°C and about 30-40°C.

28. The composition of claim 26, wherein said phase transition is inducible upon introduction of said low viscosity phase mixture into a living mammalian body.

29. The composition of claim 20, wherein said cubic phase-forming compound is selected from the group consisting of lipids, surfactants and soaps.

30. The composition of claim 29, wherein said lipid is a glyceride.

31. The composition of claim 30, wherein said glyceride is glyceryl monooleate.

32. The composition of claim 31, wherein said low viscosity phase mixture comprises by weight between about 60% and about 99.99% glyceryl monooleate, up to about 40% of an aqueous phase, and between about 0.01% and 40% of said bio-affecting catalyst.

33. The composition of claim 32, wherein said low viscosity phase mixture comprises by weight about 85% of glyceryl monooleate, about 14.87% of said aqueous phase, and about 0.13% of said bio-affecting catalyst.

34. A composition comprising at least one bio-affecting catalyst retained within a high viscosity cubic phase matrix that substantially prevents release therefrom of said catalyst for a pre-determined time period, said composition comprising by weight between about 60% and about 99.99% of glyceryl monooleate, up to about 40% of an aqueous phase, and between about 0.01%

39. The method of claim 38, wherein said composition is administered by implantation into said patient's body.

40. The method of claim 38, wherein said composition is administered by removing a component from said patient's body, exposing said component to said composition, and returning said component to said patient's body.

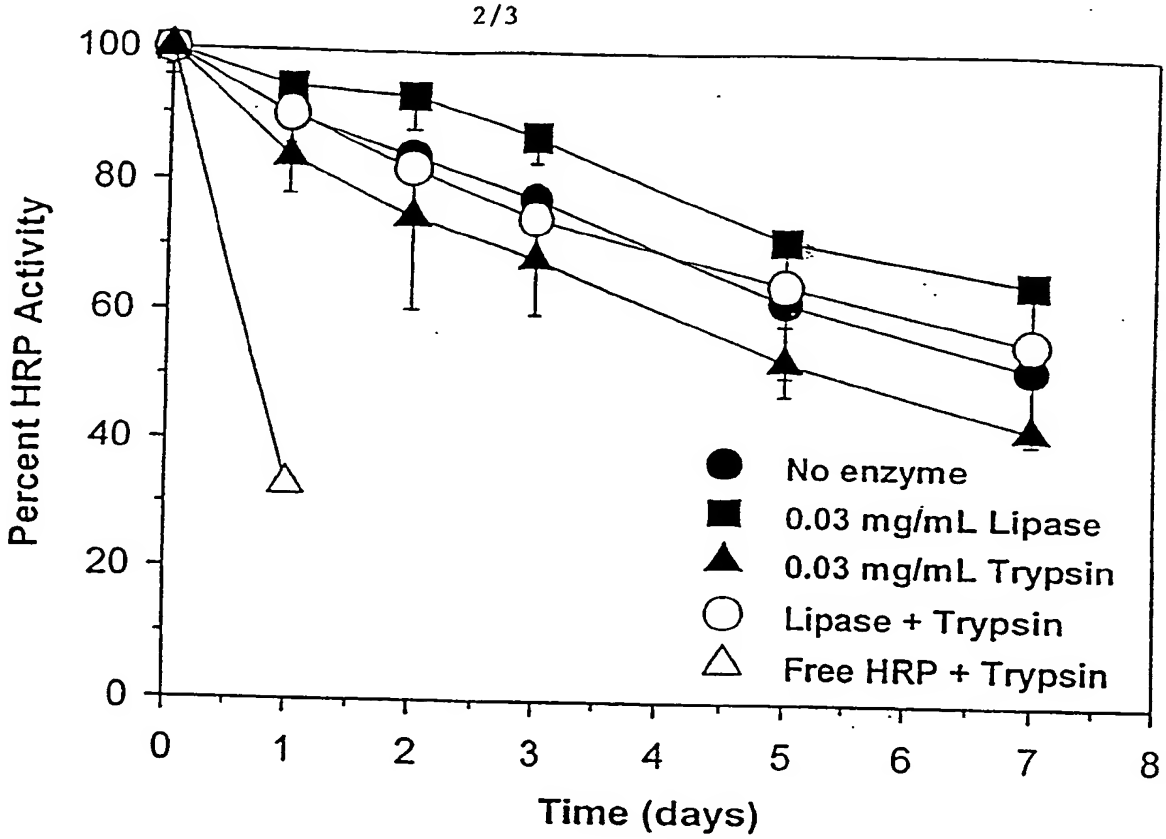
Percent HRP Activity
100
80
60
40
20
0
0
1
2
3
4
5
6
7
8
Time (days)
No enzyme
0.03 mg/mL Lipase
0.03 mg/mL Trypsin
Lipase + Trypsin
Free HRP + Trypsin

Figure 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/09262

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 9/14, 47/30
US CL : 424/486; 514/772.3, 785, 944

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/486; 514/772.3, 785, 944

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, 5,162,430 A (RHEE ET AL.) 10 November 1992, see column 3, lines 17-52, and claims.	1-40

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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